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ANAEROBIC CO-DIGESTION OF MICROALGAE AND RESIDUAL GLYCEROL RECOVERED FROM BIODIESEL: EVALUATION OF PRETREATMENT AND COD/N RATIO

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Abstract
Anaerobic digestion can be a viable alternative for the destination of effluents from stabilization ponds rich in naturally produced microalgae, without the need for concentration processes, however, there are challenges related to the rigid cell wall of microalgae and the low carbon content in its composition, to be overcome. To improve these parameters, this study comparatively evaluated hydrolytic pretreatments in microalgae from effluents from stabilization ponds, aiming to hydrolyze the cell wall of these microorganisms, for co-digestion with residual glycerol from biodiesel. In this case, glycerol is a by-product with limited applicability, acting in this scenario as a carbon supplier, improving the C/N ratio, microalgae biodegradation and biomethane production. Effluents with microalgae submitted to thermal and ultrasonic hydrolysis (for 30 and 90 minutes) were tested to assess their potential in the production of methane-rich biogas, monitored by gauge measurements and gas chromatography, respectively, in co-digestion with residual glycerol from biodiesel. The heat treatment for 30 minutes showed more satisfactory results and was replicated in a benchtop anaerobic reactor (R2), in parallel with a reactor operating untreated microalgae (R1), in a continuous feed system. The effects of pretreatment and COD/N ratio were evaluated on organic matter removal and biomethane production. R2 showed the most satisfactory effect on COD removal, resulting in up to 90% COD removed, with a theoretical biogas production of 0.52 L g^{-1} COD removed. As for the methane content contained in biogas, R1 reached percentages of up to 84% against 73% in R2.

Keywords: biodiesel, biomethane, stabilization pond, microalgae.

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Introduction

Almost all human activities require water to be accomplished, generating astonishing amounts of wastewater daily. In developing countries, about 90% of wastewater is improperly disposed (Hasan *et al.*, 2019). This problem has been studied for decades due to the imperative urgency for adequate, affordable solutions to deal with wastewater so that it is correctly disposed of in water bodies, in accordance with the legal restrictions established in every country.

The decreasing availability of freshwater reserves on earth added to the increasing needs for water (human consumption, agriculture, industrial uses etc.) make it necessary to adopt strategies to rationalize the use of water resources and reduce the negative impacts of industrial effluents (Júnior *et al.*, 2019; Chai *et al.*, 2021).

Stabilization ponds are one type of treatment for effluents widely used in tropical regions. The technology takes advantage of the warm weather and of the symbiotic relationship between algae and bacteria, where the organic matter available in the effluent is degraded by the aerobic bacteria producing ammoniacal nitrogen, phosphate, and carbon dioxide, which in turn are consumed by the algae.

Using sunlight as a source of energy, these organisms synthesize the cellular material (Cerón-Vivas *et al.*, 2018; Sarkar *et al.*, 2018) and the enrichment of nutrients caused by the symbiotic process facilitates the growth of different species of microalgae, producing a residue that is suitable for further use and/or final disposal (Hsueh *et al.*, 2007).

Microalgae or unicellular algae are microorganisms with a relatively simple structure but important biotechnological applications (Jalilian *et al.*, 2020). When participating in anaerobic digestion, they yield macro and micronutrients, in addition to providing a buffer effect (Sialve *et al.*, 2009), increasing the organic load.

As to biodegradation, the monodigestion of this substrate has a limiting feature due to disproportionality of the carbon/nitrogen (C/N) ratio. A good strategy to overcome this constraint is the co-digestion, the simultaneous digestion of two or more substrates, which rebalances the C/N ratio that is essential for the microbial consortium (Solé-Bundó *et al.*, 2019). The co-digestion also helps maintaining a favorable environment when it comes to the inhibitory effect of the ammonia that is generated in the process (Hidaka *et al.*, 2017; Lu *et al.*, 2019).

Some studies discuss the application of anaerobic digestion to obtain biogas, a renewable fuel produced by the breakdown of organic matter, pointing out that it has major advantages, because it occurs under standard ambient temperature and pressure, reaching theoretical yields of methane above 90% (Im *et al.*, 2019; Chong *et al.*, 2020).

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When it comes to renewable resources, there has been a clear movement in Brazil to turn its energy matrix more and more sustainable and less dependent on fossil fuels. This has been endorsed by governmental policies like Law No. 13.263 (Brasil, 2016), which enforced the addition of 10% of biodiesel to all the diesel commercialized in the country.

These legal requirements have undoubtedly contributed to reduce petroleum consumption and the Brazilian share on greenhouse gas emissions (Pinto *et al.*, 2012; Vieira *et al.*, 2021), but at the same time, have boosted the generation of glycerol, a by-product that represents about 10% in mass of the produced biodiesel.

Glycerol requires environmentally adequate disposal and still has a limited range of applications (Leoneti *et al.*, 2012; Colombo *et al.*, 2017; Im *et al.*, 2019; Pitt *et al.*, 2019), but some alternative treatments have been developed to allow its use/reuse, like hydrogenation to produce propylene glycol or conversion to propanediols, and the conversion into hydrogenated additives for the biofuel itself (Lacerda *et al.*, 2014; Silva *et al.*, 2017).

This research assessed the potential of hydrolytic treatments applied to effluents from wastewater stabilization ponds, as a way to improve the hydrolysis of the cell walls of microalgae – which naturally proliferate in stabilization ponds –, and then go through anaerobic biodigestion, aiming at evaluating the bench-scale rates of soluble COD removal and methane production. In addition, residual glycerol from biodiesel was also utilized as a co-substrate and carbon supplier to the system, in order to optimize the digestion of low-carbon substrates.

The study presents a viable alternative to treat effluents from wastewater stabilization ponds rich in microalgae, eliminating the need to concentrate and determine the species therein, optimizing the process and saving valuable resources, especially with respect to glycerol from biodiesel, a residue that is abundantly formed in its production process and would surely benefit from this valuable application.

Methodology

Microalgae

Microalgae were used from effluents from a stabilization (maturation) pond system. The samples were collected, refrigerated at approximately 4ºC and submitted to physicochemical characterization, without any processing for the purpose of concentration or determination of the species present. Pretreatments of the material were also carried out in the first stage (thermal and ultrasonic, for 30 and 90 min) and in the second stage of the experiment (heat pretreatment for 30 minutes), in addition to the use in the natural form.

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Glycerol
The residual glycerol used in the study came from a Brazilian biodiesel plant. Physical-chemical characterization and dilution were performed to obtain a solution with a final COD concentration around 80 g/L.

Inoculum
The inoculum used was sludge from an Internal Circulation Reactor (IC) from a sewage treatment plant at a local brewery. The inoculum sludge presented a granular appearance and was properly characterized and subjected to a specific methanogenic activity (SAM) test, using glucose as substrate and a food/microorganism ratio of 0.5.

Hydrolytic Test
The treatments were carried out with the aim of promoting the hydrolysis of the microalgae cell wall and thus making the material more accessible to the microorganisms participating in the digestion (Sialve *et al.*, 2009). The raw effluent – obtained from a maturation pond containing microalgae – was separately subjected to two types of pretreatments: ultrasonic (30 and 90 minutes) and thermal (30 and 90 minutes).

The experiment assessed both type of pretreatment and time of exposure and was based on a 2x2 factorial design. Thus, all possible combinations required only 4 trials (2^2), but each was performed twice (8 trials in total), following the recommendations of Angelidaki *et al.* (2009).

For the ultrasonic pretreatment, two samples of microalgae were kept under ultrasonic waves for 30 and 90 minutes in an ultrasonic bath using an ultrasonic cleaner (Unique Ultracleaner 1600A 40 kHz). For the thermal pretreatment, two different samples were submitted to treatment in a vertical autoclave (Marconi) for 30 and 90 minutes, at maximum pressure of 1.5 kgf/cm^2 and 120 ^{o}C.

After pretreatment, the samples were incubated in 110 mL glass vials, previously calibrated and sealed with rubber septa and aluminum fasteners, comprising 50 mL of solution and 60 mL of headspace. To the pretreated microalgae, the inoculum sludge (5 g VS/L) and 1.5 mL of glycerol solution (80 g COD/L, to improve C/N ratio) were added, obtaining a 2500 mg COD/L solution. Three controls were tested: the first containing only inoculum, the second containing only glycerol, and the third containing only effluent rich in microalgae without pretreatment.

Incubation took place in an orbital shaker (Marconi MA-420) and was maintained long enough to exhaust all available substrate for methane production, under controlled temperature (35°C) and agitation (150 rpm). The amount of biogas produced was determined using a manometer, keeping the temperature and volume of the gas phase (headspace) of the reaction flask constant, the volume of biogas produced corresponding to the increase in pressure inside the flask.

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The biogas produced was then subjected to a chromatographic analysis, using a gas chromatograph (Shimadzu GC 17A) with thermal conductivity detector (TCD).

Table 1 shows details of the multivariate factorial design used in the hydrolytic tests, carried out to evaluate the effects of the different combinations between the options chosen for type of pretreatment (A) and exposure time (B).

It is important to highlight again that all possible combinations for a 2x2 factorial design are summarized in 4 trials (2^2 trials 1 to 4), however, in this study, each trial was performed twice (trials 5 to 8), following the recommendations de Angelidaki *et al.* (2009).

Table 1. Factorial planning design to optimize the study.

Parameter	Level [-1]	Level [+1]
Type of pretreatment (A)	Thermal hydrolysis (autoclave)	Ultrasonic hydrolysis (ultrasound)
Time of exposure (B)	30 min.	90 min.

Source: Authors

<u>Reactors: Configuration and Operation</u>
Two modified benchtop reactors of the UASB type (Reactors R1 and R2) were used. Both made of PVC, with recirculation (upward speed of 0.50 m/h), and phase separator, with an internal diameter of 75 mm at the bottom and 100 mm at the top (net volume of approximately 3.40 L).

Reactor 1 (R1) was loaded with effluent containing untreated microalgae and reactor 2 (R2) with effluent containing pretreated microalgae, that is, submitted to the best pretreatment determined in the previous phase, in co-digestion with residual glycerol from the biodiesel production, fed continuously from PVC reservoirs (7 L each), kept open and under agitation and refrigerated at approximately 5°C. The average flow was 2.0 L/d and the hydraulic retention time (HRT) at room temperature of 28°C was 40h.

After the activation phase of the microbial consortium, the experimental phase of 123 days was conducted in three stages, with different COD/N ratios of the digesters (20, 40 and 70), as shown in Table 2. Microalgae (protein-rich microorganisms) represented the fraction containing high nitrogen content (Brown *et al.*, 1997; Grossmann *et al.*, 2018) and glycerol corresponded to the main carbon source. The mineral composition of the microalgae was used to meet the nutritional needs of the system, so macro and micronutrients were not added (Sialve *et al.*, 2009).

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The COD/N ratios of 20, 40 and 70 were determined based on the arithmetic mean of the COD values obtained from residual glycerol from biodiesel (main carbon source) and NTK (total Kjeldahl nitrogen) from microalgae effluent (main nitrogen source).

Table 2. Phases of the study.

Phase	Substrates	COD/N Ratio
1	Microalgae + Glyerol	20
2	Microalgae + Glycerol	40
3	Microalgae + Glycerol	70

Source: Authors

Monitoring

To ensure the stability of the system during the experiment, the reactors were monitored, with weekly analyzes of temperature, pH, chemical oxygen demand (COD), alkalinity, volatile fatty acids (VFA), total Kjeldahl nitrogen (NTK), ammonia (NH_4^+), volatile suspended solids (SSV) and total suspended solids (SST). Influent and effluent COD analyzes were performed three times a week during phases 1, 2 and 3, to evaluate the influence of the pretreatment applied to the effluent with microalgae on the removal efficiency of this parameter, in each phase of the experiment in reactors of bench.

The biogas produced in the reactors was characterized and quantified by gas chromatography (Shimadzu GC 17A chromatograph) with a thermal conductivity detector (TCD). The equipment had spitless injection mode, injection volume of 1 mL, at 40°C, helium as carrier gas, a column flow of 0.7 mL/min, oven temperature of 50°C, detector temperature of 200°C and analyzes lasting 5 minutes.

Theoretical Methane Production

The determination of the volume of biogas and consequent theoretical production of methane was performed according to Chernicharo (2007), which is based on Equation 1, where theoretically 64g of COD produces 16g of CH_4 and in STP, 0.35 L CH_4/g COD. Equation 2 shows the ratio of theoretical methane production per gram of COD removed. Equation 3 shows how the operating temperature correction factor was verified. The theoretical production of methane in the reactors was verified according to the values of COD removed.

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$ **Equation (1)**

$$V(CH_4) = COD/K_t$$ **Equation (2)**

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Where:
$V(CH_4)$ - volume of CH_4 produced (L)
COD - COD removed or converted to CH_4 (gDQO)
K_t - operating temperature correction factor (g/COD/L)

$$Kt = (P \times K) / R\,(273 + t) \qquad \textbf{Equation (3)}$$

Where:
P = atmospheric pressure (1atm)
K = COD corresponding to 1 mol of CH_4 (64g COD / mol CH_4)
R = gas constant (0.08206 atm.L / mol.°K)
t = reactor operating temperature (°C)

Statistical Analysis

Statistical tests for exploratory data analysis and assessment of results were applied in all phases of the study, using the default routines available in the software Statgraphics® Centurion XV (StatPoint., Inc).

The effect of pretreatment in each phase was evaluated by the nonparametric Mann-Whitney U test with a confidence interval of 95% ($p<0.05$), for the comparison of medians. This test was chosen because the samples were small from a statistical point of view. Each phase was evaluated separately in order to analyze the significance of pretreatment in phase 1 (COD/N=20), phase 2 (COD/N=40) and phase 3 (COD/N=70).

The effect of increasing the COD/N ratio in both reactors (R1 and R2) was evaluated using the Kruskal-Wallis test to compare medians, with the acceptance of the null hypothesis (median phase 1 = median phase 2 = median phase 3) in case the p value is less than 0.05, with a confidence interval of 95%.

Results and discussion

Characterization of inoculum and substrates

The parameters used to characterize the effluent with microalgae and the sludge used in this study are shown in Table 3.

As to the effluent with microalgae, after the treatments, the following results for the parameters total and soluble COD were obtained: (*i*) for diluted microalgae autoclaved for 30 minutes: total COD=199.20 mg/L, soluble COD=109.70 mg/L; (*ii*) for diluted microalgae autoclaved for 90 minutes: total COD=238.14 mg/L, soluble COD=175.04 mg/L; (*iii*) for diluted microalgae treated with ultrasonic waves for 30 minutes, total COD=172.63 mg/L, soluble COD=132.07 mg/L; and (*iv*) for diluted microalgae treated with ultrasonic waves for 90 minutes, total COD=281.27 mg/L, soluble COD=144.46 mg/L.

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Table 3. Parameters used to characterize the effluent with microalgae, glycerol, and sludge.

Parameter	Effluent with microalgae	Glycerol	Sludge
Ammonium	14.56 mg/L	0.896 mg/L	12.16 mg/L
TKN	20.48 mg/L	--	2430 mg/L
Total COD	264.22 mg/L	805082 mg/L	26351 mg/L
Soluble COD	10.32 mg/L	--	10 907 mg/L
VSS	50 mg/L	--	52 030 mg/L
TSS	--	--	71 385 mg/L
Alkalinity	--	301 mg/L	--
pH	--	--	6.82
SMA	--	--	0.26g COD/g.VSS

Legend: SMA - specific methanogenic activity. Source: Authors

Hydrolytic Test

The possible combinations for these parameters (i.e., A & B) are shown in Table 4 as the different trials carried out. As an example, the pair in trial 1(A=-1 & B=+1] corresponded to thermal hydrolysis (A=-1) for 90 minutes (B=+1). Similarly, the combination [+1] and [+1] in trial 4 corresponded to ultrasonic hydrolysis (A=+1) for 90 minutes (B=+1).

Table 4. Combination of levels and results of the trials

Trials	Combination of Levels		Results (mL of total CH_4)
	A	B	
1	[-1]	[-1]	20.6
2	[-1]	[+1]	22.8
3	[+1]	[-1]	21.1
4	[+1]	[+1]	18.6
5	[-1]	[-1]	19.9
6	[-1]	[+1]	24.7
7	[+1]	[-1]	20.6
8	[+1]	[+1]	17.2

Source: Authors

The experimental results show that the levels A[-1]/B[+1](heat treatment for 90 min) yielded a higher amount of methane in the produced biogas. However, the Pareto diagram and the graph of main interactions (see Figure 1) show that only the variable "pretreatment" was statistically significant (95% confidence interval) whereas "time of exposure" did not present a similar

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behavior. The maximum methane volumes obtained for the control samples were: 5.6 mL of CH_4 for the inoculum-only control, 18.2 mL for the glycerol-only control, and 19.9 mL for the microalgae effluent control.

The main effect of the pretreatment together with the secondary effect of the variables influenced the increase in methane production, as shown in the Pareto diagram (Figure 1, left). This was confirmed by the graph of the main interactions (Figure 1, right), where the type of pretreatment ("treatment" in Figure 1) appeared as a very inclined slope, as opposed to the line indicating the effects of factor "time of exposure", which tended to be more horizontal, demonstrating very little influence on methane production.

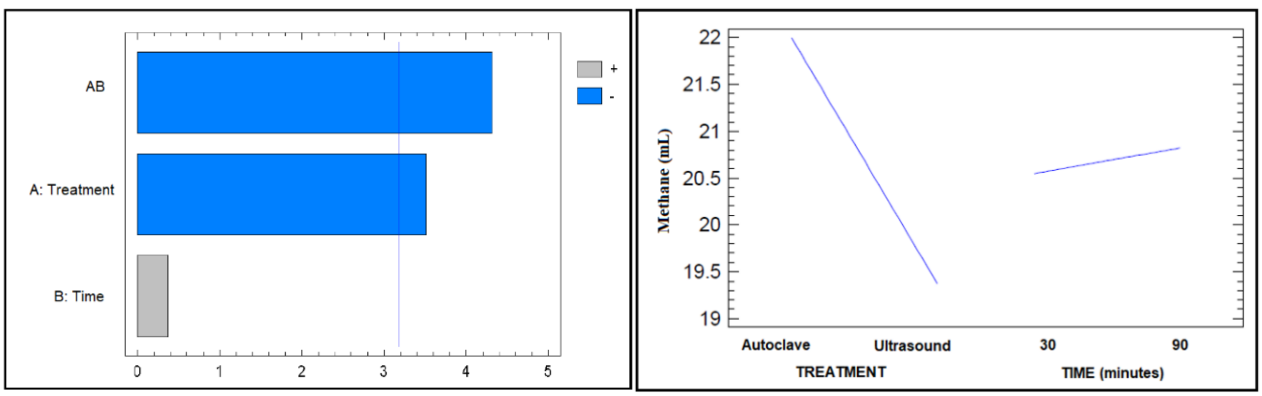

Figure 1. Pareto diagram regarding methane production (left) and main effects of assessed variables (right).

According to the statistical results, the best pretreatment was thermal hydrolysis for 30 minutes, which was then chosen to be tested in the bench reactor (second stage of the experiment), in order to compare the anaerobic co-digestion of treated and untreated microalgae with glycerol.

According to Passos and Ferrer (2015), who evaluated the impact of pre-heat treatment time on Oocystis microalgae, under pressure and for 15 and 30 minutes, the increase in pretreatment time did not favorably influence the efficiency of methane production.

Studies carried out with several species of microalgae to constitute the biomass to be degraded, submitted to heat pretreatments in identical periods, showed that the microalgae species influences the efficiency of the heat pretreatment and consequently the production of methane during anaerobic biodegradation (Alzate *et al.*, 2012; Alzate *et al.*, 2014). The present study worked with microalgae species naturally produced in the effluent of stabilization ponds and did

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not perform microalgae concentration procedures or identification tests of these species, however, the results can be considered satisfactory, since it was possible to achieve production results of biogas rich in methane, with optimization in the use of high-cost procedures and analyses.

Operational parameters for bench reactors

During the experiment, as the COD/N ratio was increased, the pH tended to drop, due to the increase in the organic load, which boosted the production of volatile fatty acids (VFAs) (Chernicharo, 1997). In phases 1 and 2, alkalinity and pH remained at levels favorable to digestion, even without the addition of sodium bicarbonate as a buffer. Alkalinity values ranged between 149 and 233 mg $CaCO_3$/L, and VFAs between 41 to 134 mg/L, with the VFA/Alkalinity ratio remaining below 0.3 (Bayr *et al.*, 2014; Kim and Kang, 2015). In phase 3, the buffer had to be added to maintain the alkalinity and ideal conditions for anaerobic digestion, as a result of an increase in the organic load concentration, which caused a sudden drop in pH.

Influence of pretreatment and COD/N ratio on COD removal efficiency

Figures 2, 3 and 4 show the influence of pretreatment on the removal of soluble COD in phases 1, 2 and 3, in both reactors (R1 and R2). In phase 2, a value of $p = 4.4 \times 10^{-4}$ was obtained, a condition for rejecting the null hypothesis, considering that, in this phase, the medians of R1 and R2 are statistically different. Therefore, the pretreatment of the effluent with microalgae positively influenced the removal of soluble COD (Figure 3), when it was observed that the removal values were higher in R2. It is worth mentioning that the difference between the 1st and 3rd quartiles of R2 is smaller than that of R1, which demonstrates that there was greater stability of R2 in this phase. In phases 1 and 3, p values were greater than 0.05, therefore, there was no significant difference between the reactors in the removal of soluble COD in these phases.

Soluble COD removal efficiencies of 50-84% were obtained, with volumetric organic loads varying between 0.21 and 0.75 kg COD $m^{-3}d^{-1}$. Ras *et al.* (2011) worked with anaerobic digestion of *Chlorella vulgaris*, with 28 days of hydraulic retention time (HRT), obtaining COD removals of 51%. Lee and Kim (2018) demonstrated that the conversion efficiency of biomass residues of the *Braunii* strain of microalgae with glycerol was 80% above theoretical methane production. Meneses-Reyes *et al.* (2017) worked with the co-digestion of microalgae after oil, glycerol, and poultry litter extraction in different proportions, obtaining a COD removal of 91.02%. The present study obtained COD removal results above the average encountered in the examined literature.

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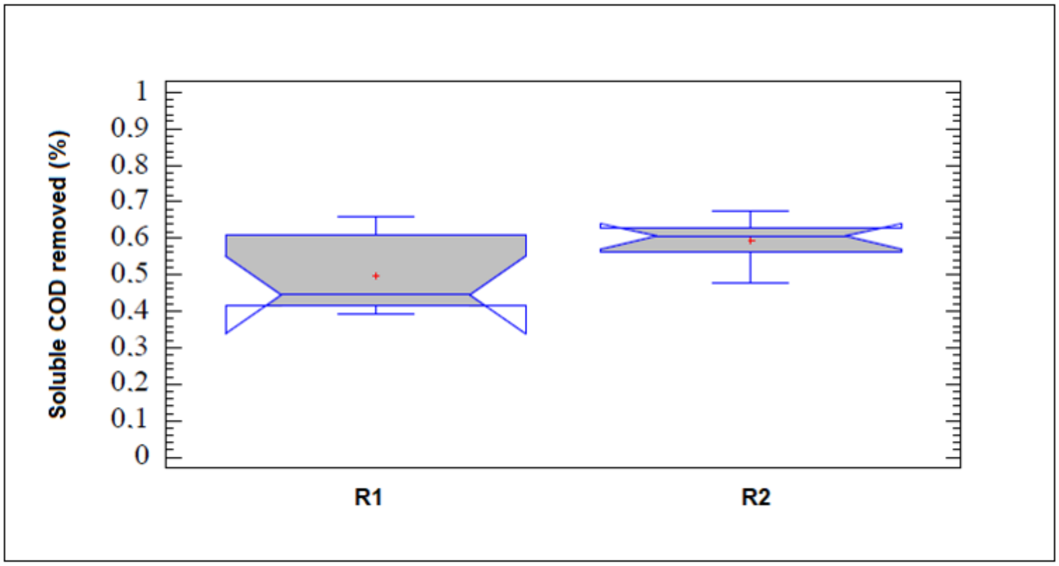

Figure 2. Influence of pretreatment in COD removal in Reactor 1 (R1) and Reactor 2 (R2) in phase 1.

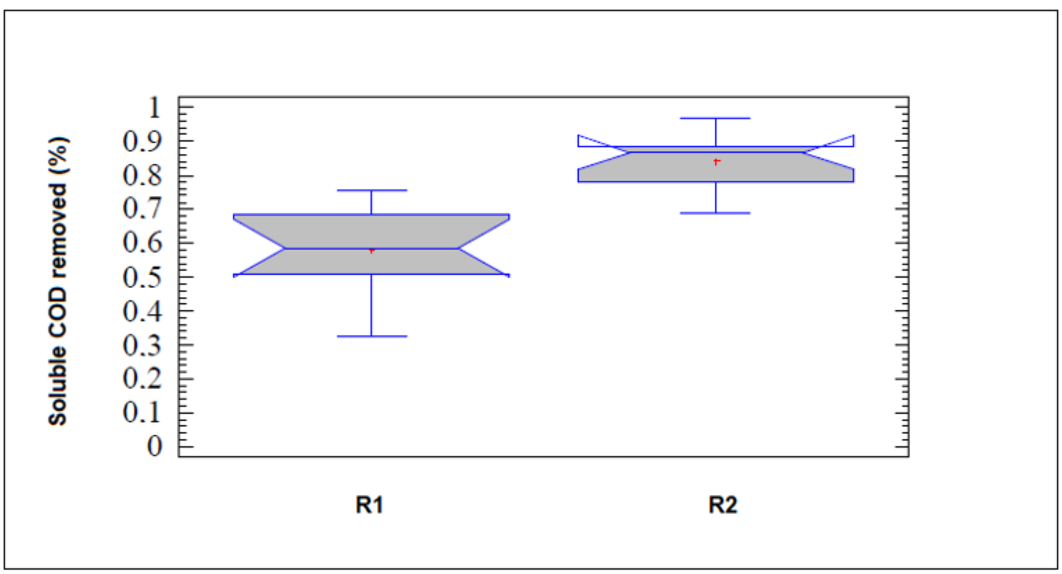

Figure 3. Influence of pretreatment in soluble COD removal in Reactor 1 (R1) and Reactor 2 (R2) in phase 2.

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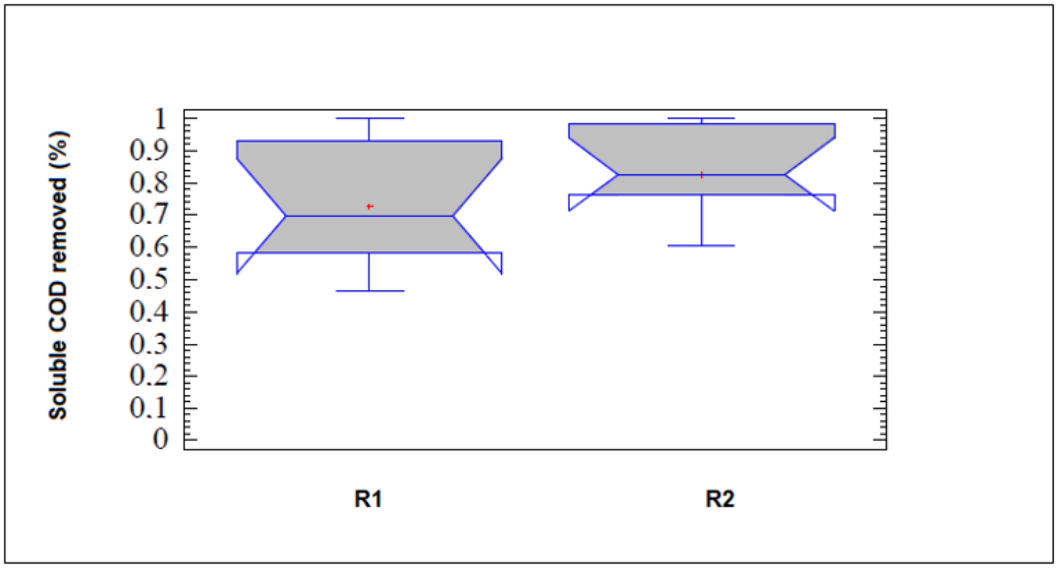

Figure 4. Influence of pretreatment in soluble COD removal in in Reactor 1 (R1) and Reactor 2 (R2) in phase 3.

Influence of the COD/N ratio on the removal of organic matter

According to the statistical test used, in both reactors, the increase in the COD/N ratio had a significant influence on the removal of organic matter ($p<0.05$).

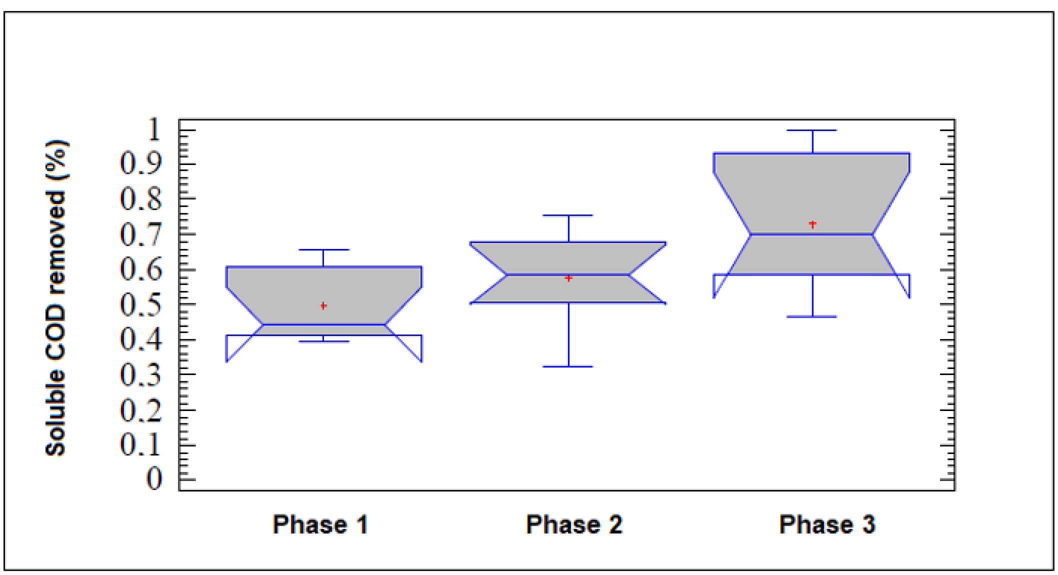

Figure 5. Influence of COD/N ratio on soluble COD removal (%) in Reactor R1.

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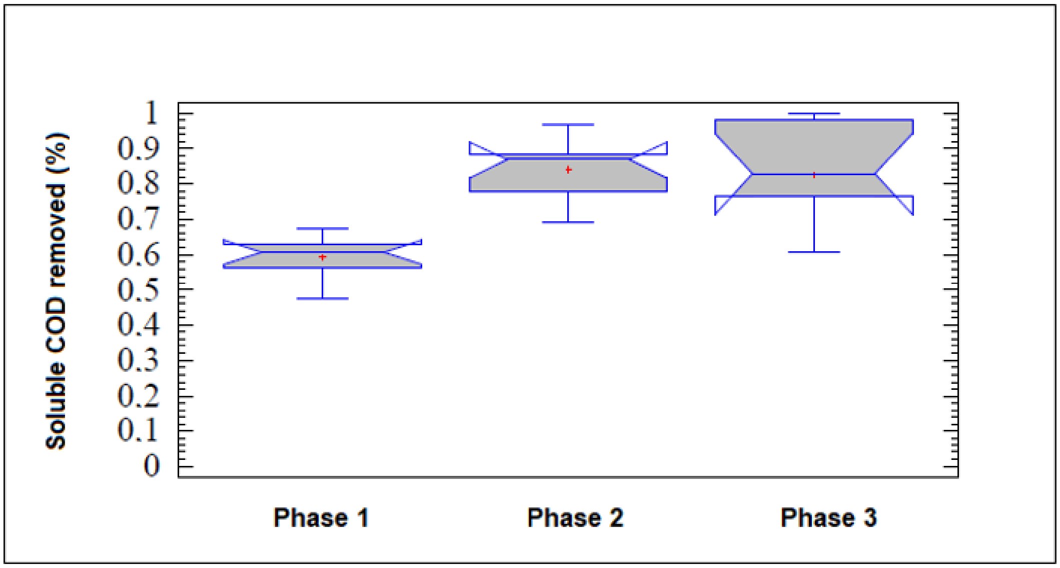

Figure 6. Influence of COD/N ratio on soluble COD removal (%) in Reactor R2

In R1 ($p=0.034$), the null hypothesis was rejected, as there was a significant difference between the medians of phases 1 and 3, with phase 3 (COD/N = 70) being the one that presented the best results in terms of COD removal soluble. In R2 ($p=9.36x10^{-4}$), the null hypothesis was also rejected, as there was a significant difference between phases 1 and 2 and between phases 1 and 3.

In both reactors, the statistical analysis showed that the increase in the COD/N ratio influenced the removal of organic matter. When evaluating the conditions found in R1, the removal of soluble COD had the best results in phase 3 (COD/N=70). In R2, although no significant difference was observed between phases 2 and 3, phase 2 can be considered as the one with the best performance, taking into account the greater stability observed in the pretreatment evaluation in this phase (COD/N = 40).

Few studies in the literature relate the direct influence of the COD/N ratio on the removal of organic matter in anaerobic biodegradation systems, with the C/N ratio being more common. A study by Xie *et al.* (2012) correlated the COD/N parameter with denitrification and methanization rates during anaerobic digestion, reporting that, for COD/N>53, the ammonia generation pathway simultaneously favors methane production and denitrification.

According to Sumardiono *et al.* (2013), the ideal COD/N ratio for the occurrence of anaerobic digestion is between 50 and 142, not in line with the study in question, in which in phase 2

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(COD/N=40) it presented better performance, in the co-digestion of effluent with heat-treated microalgae and residual glycerol from biodiesel.

Concentration of methane in biogas in Reactors R1 and R2 in all phases

Considering the theoretical methane production, the volume of biogas accumulated in the reactors was 0.45 L g^{-1} of COD removed in R1, and 0.52 L g^{-1} of COD in R2. In percentage terms, the biogas in R1 had 84% of methane and R2, 73%, results close to those found in other studies (Park and Li, 2012; Mahdy *et al.*, 2015). R1 produced slightly higher percentages of methane when compared to R2, throughout the experimental phase (Figure 7).

Solé-Bundó *et al.* (2019), who worked with pretreatment of microalgae from wastewater treatment systems, using heat treatment at 75 °C for 10 hours, for co-digestion with primary sludge, emphasized that microalgae that received pretreatment showed low methane yield in anaerobic digestion (0.16 L CH_4/g SV), when digested in anaerobic conditions, and this was probably attributed to the presence of species with resistant cell walls detected by microscopic analysis, which were probably not broken during treatment. According to Wang *et al.* (2017), heat treatment can also induce the so-called Maillard reaction, which triggers the formation of several compounds that can be toxic to methanogenic archaea.

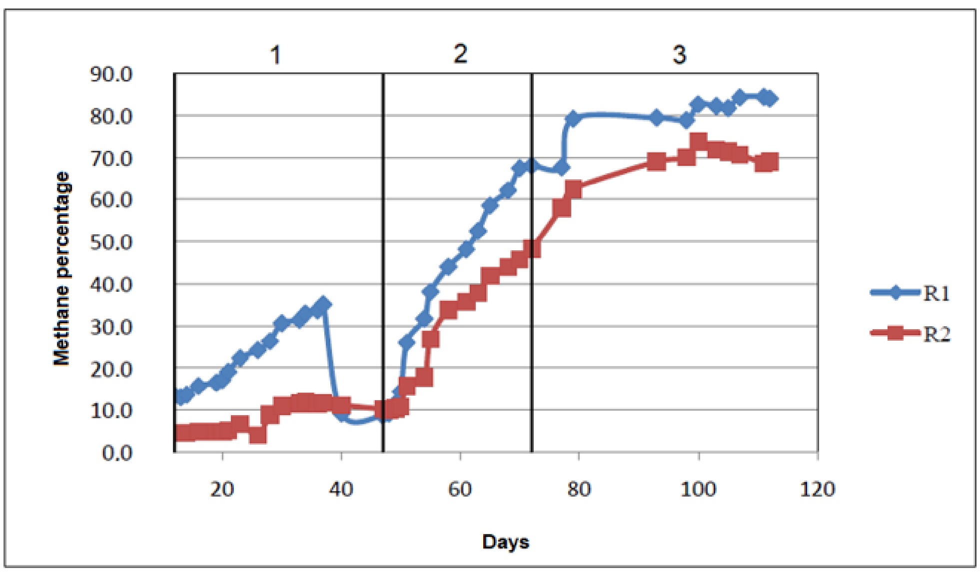

Figure 7. Percentage of methane in biogas as a function of days in Reactors R1 and R2, considering the phases 1, 2, and 3 of the experiment

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The percentage values of methane found in this work were higher than those found by Solé-Bundó *et al.* (2019), who obtained an average percentage of 65.5% of methane in the biogas, anaerobically digesting *Chorella sp*. of domestic sewage, Gonzaléz-Fernandez *et al.* (2016) who obtained an average percentage of 67.2 to 69.8% working with *Chlorella sp*., *Scenedesmus obliquus*, *Chlamydomonas reinhardtii* from domestic sewage, Wieczorek *et al.* (2015) who obtained an average percentage of 69.2 to 71.9% working with *Chlorella vulgaris*, wastewater from the paper production industry *Scenedesmus sp*. of domestic sewage and the percentages found by Park and Li (2012), which ranged from 33 to 69%, in the anaerobic co-digestion of algal biomass residue and oil, grease and fat residues.

The biogas production observed in this study indicated that there was a stable equilibrium during anaerobic digestion, in which the production of biogas with high percentages of methane implied a lower need to purify it (Park and Li, 2012). This percentage of methane in the biogas is an indication that the forms of inhibition of methanogenic activity in the microbial consortium were very low or negligible (Park and Li, 2012).

Conclusions

This study demonstrated that the hydrolytic pretreatment applied to effluents of wastewater stabilization ponds to boost the hydrolysis of the cell wall of microalgae was satisfactory in improving anaerobic biodegradation, with favorable results with regards to soluble COD removal and the percentage of methane production in biogas, when compared to other studies. The addition of residual glycerol co-substrate from biodiesel contributed to the improvement of some aspects of the anaerobic digestion process, such as carbon supply and balance of organic load.

Finally, the high production of methane (in percentage terms) from microalgae biomass present in effluents from a stabilization pond, with the residual glycerol substrate from biodiesel production, allows the generation of bioenergy in a sustainable way, from sources that would normally be discarded without any use. Furthermore, in the process here proposed, the employment of microalgae biomass (naturally present in effluents from stabilization ponds), without increasing concentration or identifying species, will save time and production costs.

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